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(54) Title: TISSUE ENGINEERING SCAFFOLDS

(57) Abstract: A process for preparing a scaffold of biocompatible polymer which comprises placing a composition comprising the polymer in a mould possessing one or more voids therein, said mould being a negative of the desired shape including a designed architecture and dimensions of the scaffold, causing the polymer to acquire the shape of the mould and causing pores to be formed in the polymer, and removing the mould without affecting the polymer.

TISSUE ENGINEERING SCAFFOLDS

This invention relates to tissue engineering scaffolds.

Tissue engineering is a new multidisciplinary field that involves the development of biological substitutes that restore, maintain or improve tissue function. This field has the potential of overcoming the limitations of conventional treatments by producing a supply of organ and tissue substitutes biologically tailored to the patient.

Tissue engineering involves growing the relevant cell(s) in the laboratory into the required organ or tissue. However, unaided cells lack the ability to grow in favoured orientations and thus define the anatomical shape of the organ and tissue. Instead, they randomly migrate to form a two dimensional layer of cells. Thus, three dimensional (3D) tissues are required and this is achieved by the use of 3D scaffolds, which act as substrates for cellular attachment. Scaffolds are required to 1) have porosity, generally interconnecting, so as to allow tissue integration and blood vessel colonisation, 2) be made of a biodegradable or bioresorbable material so that tissue can eventually replace the scaffold as it degrades, 3) have appropriate surface chemistry to favour cell attachment, proliferation and differentiation, 4) possess adequate mechanical properties to match the intended implantation site and 5) be easily fabricated into a variety of shapes and sizes. In particular, the pore size of the scaffold has been identified as critical for the successful growth of tissues. An average pore size range of 200 to 400 μm has been shown as optimum for the growth of bone tissue.

Biodegradable and bioresorbable polymers and ceramics have been used as the material to make the scaffolds. The majority of the work has focussed on polymers since ceramic scaffolds have been aimed mostly at bone tissue engineering. The polymers which have been used are synthetic (e.g. polylactic acid and polyglycolic acid, FDA approved polymers used for sutures and orthopaedic fixation screws), or natural (e.g. collagen, an abundant protein present in the connective tissue

of mammals which is FDA approved - the collagen can be from cow hide and used to correct skin contour defects).

Several techniques have been developed to produce tissue engineering scaffolds from biodegradable and bioresorbable polymers. For synthetic polymers, these are usually based on solvent casting-particulate leaching, phase separation, gas foaming and fibre meshes. For natural collagen scaffolds, these can be made by freezing a dispersion/solution of collagen and then freeze-drying it. Freezing the dispersion/solution results in the production of ice crystals that grow and force the collagen into the interstitial spaces, thus aggregating the collagen. The ice crystals are removed by freeze-drying which involves inducing the sublimation of the ice and this gives rise to pore formation; therefore the water passes from a solid phase directly to a gaseous phase and eliminates any surface tension forces that can collapse the delicate porous structure. These techniques are, however, generally dependent on a pore generator to form the pores within the scaffold, e.g. salt particles, liquid-liquid phase separation, gas bubble evolution or ice crystals. However, the distribution of pores and fibre bonding locations cannot be precisely controlled and consequently these techniques are unable to ensure reliable interconnection and distribution of pores within the scaffold. Consequently, these techniques cannot produce complicated internal features, like channels, that can act as an artificial vascular system which would favour the growth of blood vessels and could sustain the cell growth deep into the scaffold. In this connection it should be borne in mind that as a general rule the parenchymal or supportive cells of vascularised tissues *in vivo* (except cartilage) are no further than 25-50μm from the nearest blood vessel.

Solid Freeform Fabrication (SFF) (also known under the generic name of Rapid Prototyping (RP)) technologies have the potential to significantly impact on tissue engineering by producing scaffolds with tailored architectures and thus overcome the limitations of the current fabrication techniques. SFF processes involve producing three-dimensional objects directly from a computer-aided design model using layered manufacturing strategies. They are capable of delivering complex shapes exhibiting intricate internal features directly from computer-

11. A process according to claim 10 wherein the particles are electrical and electrical particles are also applied to the mould.

12. A process according to any one of claims 1 to 9 wherein electrical or magnetic particles are applied to the mould.

13. A process according to any one of the preceding claims wherein the composition is frozen while in the mould to acquire the shape of the mould.

14. A process according to claim 13 wherein the collagen is frozen to a temperature from -20°C to -80°C.

15. A process according to any one of the preceding claims wherein the mould is removed by the addition of a solvent therefor which is a non solvent for the biodegradable polymer.

16. A process according to claim 15 wherein the mould is dissolved in a polar solvent which is a non solvent for collagen.

17. A process according to claim 16 wherein the polar solvent is ethanol, 2-propanol, propanone, water or an aqueous ethanolic solution.

18. A process according to any one of claims 15 to 17 wherein the solvent for the mould is removed from the collagen by critical point drying using liquid carbon dioxide.

19. A process according to any one of the preceding claims wherein the scaffold is provided with a laminated or mosaic structure, with layers or regions having different chemical compositions.

20. A process according to any one of the preceding claims wherein the mould is shaped such that the external shape of the scaffold has the gross shape of the organ for which it is to act as a replacement.

21. A process according to any one of the preceding claims wherein the scaffold comprises one or more conduits either for the growth of peripheral nerves, blood vessels, connective tissue and/or highly vascularised vital organs, and/or for the provision of nutrients for such growth.

22. A process according to any one of the preceding claims wherein the mould is made of cholesterol.

23. A process according to any one of the preceding claims wherein the mould is made with the aid of a support of polyethylene glycol.

24. A process according to claim 1 substantially as described in either of the Examples.

25. A scaffold of biocompatible polymer whenever prepared by a process as claimed in any one of the preceding claims.

26. A scaffold of biocompatible polymer obtainable by a process claimed in any one of claims 1 to 24.

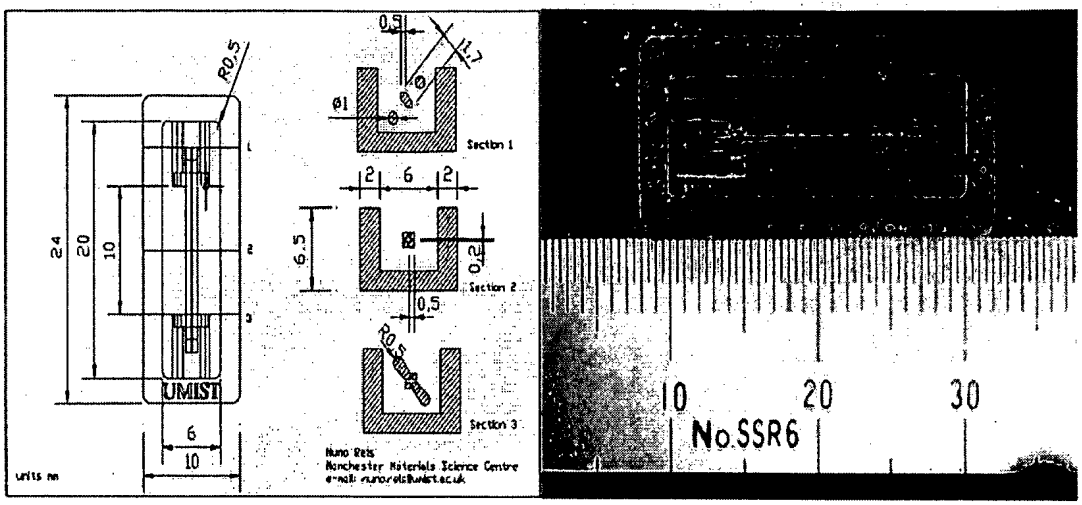

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Figure 1

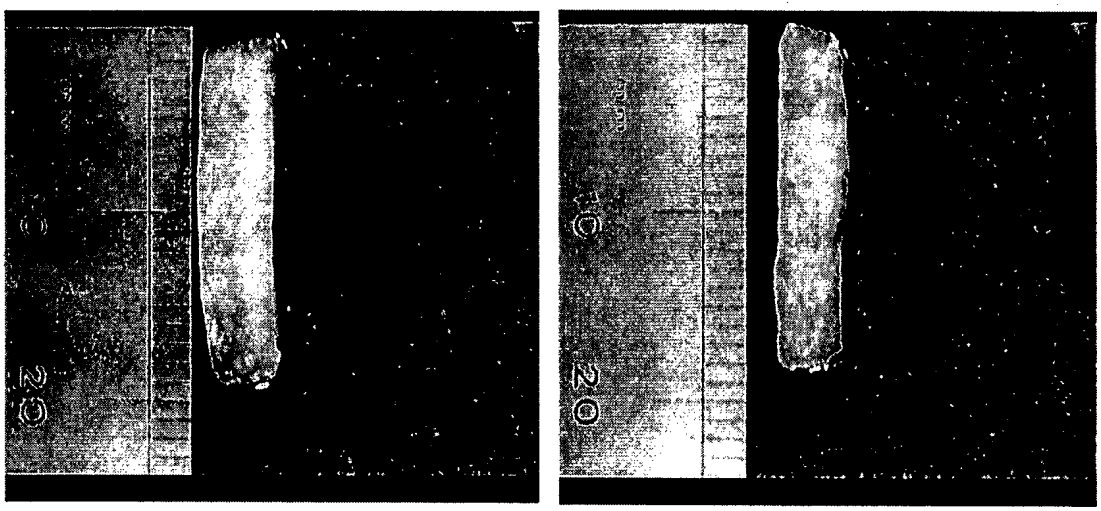

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Figure 2

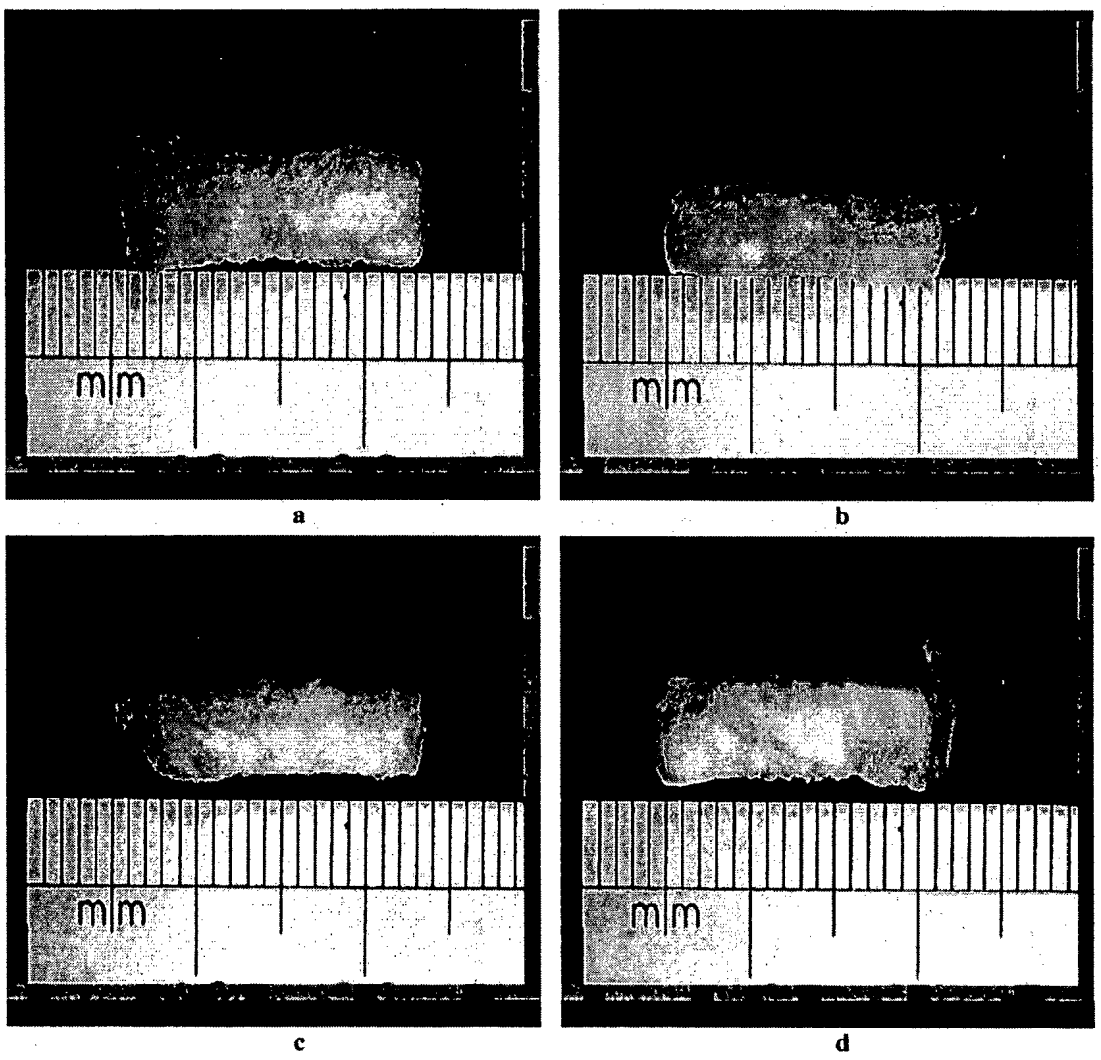

Figure 3

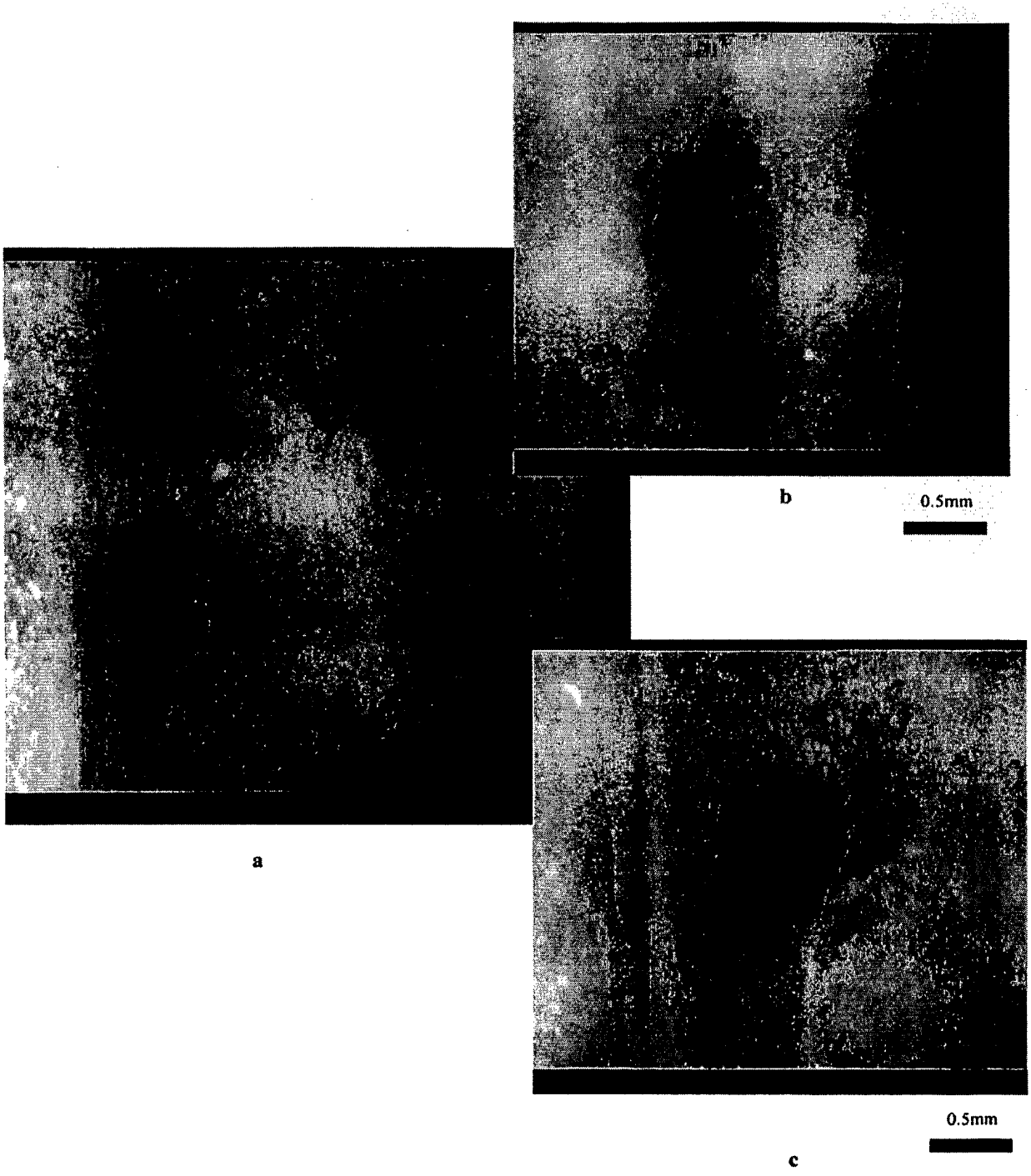

Figure 4